

General Approach to Toxicology Cases

1 Scope

This procedure provides general guidelines for the analysis of toxicology cases in the Chemistry Unit of the FBI Laboratory. These guidelines are based on recommendations set forth in a number of reference documents such as:

- *Society of Forensic Toxicologists (SOFT)/American Academy of Forensic Sciences (AAFS) Forensic Toxicology Laboratory Guidelines*
- *Drug Abuse Handbook*
- *Tietz Handbook of Clinical Chemistry*
- *Poison Detection in Human Organs*
- *Introduction to Forensic Toxicology*
- *Principles of Forensic Toxicology, Handbook of Analytical Toxicology*
- *FBI Laboratory Quality Assurance Manual*
- *FBI Laboratory Operations Manual*
- *Chemistry Unit Quality Assurance and Operations Manual*
- *American Society of Crime Laboratory Directors / Laboratory Accreditation Board (ASCLD/LAB) Accreditation Manual*
- *International Organization of Standardization (ISO) / International Electrotechnical Commission (ISO/IEC) 17025 Requirements*
- *ASCLD/LAB-International Supplemental Requirements*
- *American Board of Forensic Toxicology, Inc. (ABFT) Laboratory Accreditation Checklist*

2 Equipment/Materials/Reagents

Not applicable.

3 Standards and Controls

Not applicable.

4 Sampling and Sample Selection

The most common toxicology specimens, blood, vitreous humor and urine, are liquid. Before sampling, containers will be inverted, swirled or vortexed to ensure homogeneity. If a blood sample is clotted, clots may have to be destroyed before sampling. This can be performed using a ground glass clot buster or with a disposable plastic clot buster. When blood samples are very viscous and cannot be pipeted accurately, they may be sampled by weight. When this occurs, the weight will be documented in case notes to the nearest 0.01 gram. When blood must be weighed for a quantitative examination, this will be considered in the uncertainty calculations. Additionally, concentrations will be reported in w/w instead of in w/v.

Blood samples may be received in multiple tubes. When blood is collected at the same time, from the same location, it may be considered the same item number upon initial check-in. These items will be further marked A, B, etc. in the Chemistry Unit, and each chemist will document in the case notes which tube was sampled from for each examination.

Tissue, gastric, and food samples are not always homogenous. Therefore, they are typically homogenized in a blender before analysis. It is not always feasible to homogenize the whole item, and often, some will be kept in the original form should additional testing be required. To prepare a homogenate, a portion is removed and weighed to the nearest gram. An equal amount of water (by weight) is added before blending to create a 1:1 (also called a two-fold) homogenate. Homogenates will be sampled by weight to the nearest 0.01 gram, and quantitative results will be reported in w/w.

When the total amount of a drug or poison in a gastric sample or food sample is needed for interpretative reasons, the whole item will be homogenized before analysis.

5 Procedure

5.1 Sample Collection and Receipt

The proper selection, collection, and submission of biological and other specimens for toxicological analyses is of paramount importance for scientifically sound interpretation of analytical results. While there are recommended minimum amounts of specific specimens desired to accomplish routine toxicological examinations (Tables 1 and 2), specimen amount is often limited. In these cases, the type and amount of specimen submitted may dictate the analyses that are performed.

For the analysis of blood for certain drug classes (e.g., cocaine, ethanol, cyanide), it can be advantageous if the specimen is mixed with a preservative in order to enhance stability of the analytes. This can be accomplished by using specimen tubes containing preservatives. For most toxicology cases, the preferred collection tube is a grey-top Vacutainer[®], containing a mixture of sodium fluoride and potassium oxalate. If a blood specimen is not properly preserved, it is important to know what effect that this may have on the analyte(s) of interest.

Each specimen should be identified as to type. For blood, the anatomical site of collection should be stated. Specimens collected from a living person should be labeled with the time and date of collection. Specimens should always be labeled with the donor's name.

Table 1: Minimum Specimen Requirements for Postmortem Cases

Specimen:	Amount:
Heart Blood ¹	10 mL
Peripheral Blood ¹	5 mL
Urine, Gastric Contents, Vitreous Humor, and Bile ²	All
Kidney ²	50 g
Brain and Liver ²	100 g

¹Blood is the preferred specimen for most postmortem analyses.

²For routine postmortem cases, all specimens are not required. Testing of blood and vitreous humor or blood and urine is often sufficient.

Table 2: Minimum Specimen
Requirements for Human Performance Cases

Specimen:	Amount:
Blood ³	10 mL
Urine	20 mL

Biological specimens should be submitted to the FBI Laboratory under proper seal and with appropriate warning labels. FBI cases should be submitted individually and under a single communication. Specimens should be submitted in a refrigerated or frozen condition. Upon receipt of the evidence, a chain-of-custody will be started. For more detail on these procedures, consult the *FBI Laboratory Quality Assurance Manual*, the *FBI Laboratory Operations Manual*, and the *Chemistry Unit Quality Assurance and Operations Manual*.

After assignment of a toxicology case, an Examiner or Chemist will inventory the evidence to document the type of specimen(s) received, any labeling present on the specimen containers, the specimen amounts, and any damage to or leaks from the containers. Additionally, the specimen containers will be labeled with the item number and the Laboratory number.

5.2 Specimen Storage

Due to the nature of biologicals, specimens are kept refrigerated or frozen when not under active examination. A refrigerator and a freezer are located in the main Chemistry Unit Evidence Storage Room.

5.3 Analytical Schemes for Toxicology Testing

Forensic toxicological examinations are conducted on a variety of specimens for a wide range of drugs and other substances. Toxicological examinations begin with a review of the case history. Professional judgment is used to determine the sequence of assays that will be performed for a given case. Generally, cases of suspected drug-related homicide will include a blood ethanol analysis and a standard drugs-of-abuse screen with confirmation of any relevant findings. Fatalities involving motor vehicle drivers will usually require a blood ethanol determination and a more comprehensive screen for recreational and prescription drugs. Relevant positive findings are

³Blood is the *only* specimen that permits determination of whether an individual may have been under the influence of a drug or chemical at the time of collection.

usually quantitated in the blood. Suspected drug-facilitated sexual assault (DFSA) cases may call for routine screens of urine for recreational drugs, alcohol, and other central nervous system depressants (e.g., benzodiazepines and barbiturates), but targeted screens for popular DFSA drugs (e.g., GHB, flunitrazepam, chloral hydrate) may also be necessary. Suspected poisoning cases without an alleged poison may call for a review of medical records to guide the analytical scheme. These cases may require an extensive general unknown approach which may include screening for volatile chemicals, cyanide, pesticides, etc.

When vitreous humor is the only specimen submitted in a post mortem investigation, testing will typically be limited to ethanol and other low molecular weight volatiles.

5.4 Analytical Schemes for Screening Tests

Most analytical schemes begin with the use of screening protocols for classes of drugs or poisons. Commonly employed toxicological screening techniques include, but are not limited to, immunoassays, solvent extractions, solid phase extractions, headspace analysis, thin layer chromatography, color spot tests, or the use of selective detectors on gas chromatographs. Table 3 contains a list of analytes commonly screened for in the FBI Laboratory using immunoassay, headspace gas chromatography, and common drug screens.

Table 3: Analytes Routinely Screened for in Blood and Urine in Toxicology Cases

<i>Headspace gas-chromatography/mass spectrometry</i>			
ethanol	acetone	methanol	isopropanol
<i>Immunoassay</i>			
11-nor-9-carboxy Δ^9 THC			
<i>UPLC/HRMS (ultra performance liquid chromatography/high resolution mass spectrometry)</i>			
alprazolam	flunitrazepam	oxymorphone	benzoylecgonine
α -hydroxyalprazolam	flurazepam	noroxycodone	cocaine
α -hydroxymidazolam	lorazepam	oxycodone	cocaethylene
α -hydroxytriazolam	midazolam	normorphine	ecgonine methyl ester
clonazepam	oxazepam	morphine	
7-aminoclonazepam	phenazepam	hydromorphone	
chlordiazepoxide	temazepam	norcodeine	
diazepam	zolpidem	6-acetylmorphine	
nordiazepam	zaleplon	dihydromorphone	
desalkylflurazepam	zopiclone		
7-aminoflunitrazepam			
<i>Acid/Neutral Drug Screen</i>			
acetaminophen	citalopram	lidocaine	phenobarbital

amobarbital	cyclobenzaprine	meprobamate	phenytoin
bupropion	diphenhydramine	methadone	propoxyphene
butalbital	ibuprofen	mirtazapine	secobarbital
carbamazepine	ketamine	naproxen	theophylline
carisoprodol	lamotrigine	pentobarbital	
<i>Alkaline Drug Screen</i>			
7-OH-quetiapine**	diltiazem	methamphetamine	oxymorphone
amitriptyline	diphenhydramine	methylone	paroxetine
amphetamine	doxepin	metoprolol	phencyclidine
brompheniramine	doxylamine	mirtazapine	phenylephrine**
buprenorphine	duloxetine	morphine	promethazine**
bupropion	ecgonine methyl ester	norbuprenorphine**	propoxyphene
chlorpheniramine	ephedrine	norchlorcyclizine**	propranolol
chlorpromazine	fentanyl	nordoxepin	pseudoephedrine
citalopram	fluoxetine	norfentanyl**	quetiapine
clomipramine	hydrocodone	norfluoxetine	scopolamine
clozapine	hydromorphone	norhydrocodone**	sertraline
cocaethylene	hydroxyzine	norketamine**	strychnine
cocaine	imipramine	normeperidine	tapentadol
codeine	ketamine	noroxycodone**	tetrahydrozoline**
cyclobenzaprine	MBDB	norpheniramine**	thioridazine
desmethylocyclobenzaprine**	MDA	norpropoxyphene	tramadol
desipramine	MDEA	norquetiapine**	trazodone
desmethylocitalopram**	MDMA	norsertraline	trimipramine
desmethyloclozapine**	MDPV	nortriptyline	venlafaxine
desmethyltapentadol**	meperidine	norvenlafaxine**	verapamil
dextromethorphan**	mephedrone	oxycodone	ziprasidone
dextrorphan	methadone		zolpidem

**These analytes have either only been validated in urine or are only recovered at meaningful concentrations in urine.

The purpose of screening techniques is to rule out the presence of analytes that are detectable by these techniques, or to indicate when further testing may be warranted. Screening techniques should have minimum detection limits for analytes of interest that will include therapeutic concentrations for drugs and lethal concentrations for chemicals. The selection of the screening technique(s) utilized will depend upon the case history, the available specimen, current technology, and the Examiner's professional judgment. If the goal of the analysis is to screen a blood sample

for common drugs and metabolites, the following screening techniques might be applicable: ethanol analysis, immunoassays, UPLC/HRMS screen, alkaline drug screen, acid/neutral drug screen. If the goal of the analysis is to screen a blood sample for poisons, the following analytes might be targeted: volatiles, cyanide, metals, pesticides, and carbon monoxide. Detailed instructions for these assays may be found in the Toxicology Subunit Standard Operating Procedures Manual.

5.5 Analytical Schemes for Confirmatory Tests

As a general matter of scientific and forensic principle, the detection of drugs and other toxins are confirmed (whenever possible) by a second technique based on a different chemical principle. Generally, the confirmatory test for the target analyte is more specific than the first assay. The confirmatory test will always include analysis of positive and negative controls for the analyte of interest.

When a screening technique indicates the possible presence of a drug or chemical in one biological specimen (e.g., urine), confirmation of the identity of the analyte in a second specimen from the same individual (e.g., blood) is acceptable, as is confirmation of a second aliquot of the same specimen.

Whenever possible and practical, the use of mass spectrometry is recommended for the confirmation. An immunoassay will never be used to confirm the results of another immunoassay since analytes that cross-react with one assay may cross-react in the second assay.

5.6 Analytical Schemes for Quantitations

Quantitation is performed on analytes that are important to a case (as determined by the case history, specimen volume, and the derived interpretive value used in assessing the toxicological significance). For a more detailed discussion on toxicological quantitations see the *Guidelines for Toxicological Quantitations* standard operating procedure (Tox 101).

Table 4 contains a list of analytes that have been validated for quantitation in blood samples.

Table 4: Analytes Routinely Quantitated in Blood in Toxicology Cases

6-acetylmorphine	codeine	ketamine	oxycodone
7-aminoclonazepam	cyclobenzaprine	lorazepam	oxymorphone
7-aminoflunitrazepam	desalkylflurazepam	MDA	paroxetine
acetaminophen	desipramine	MDEA	PCP
acetone	desmethyflunitrazepam	MDMA	pheniramine
α -hydroxyalprazolam	dextromethorphan	MDPV	phenobarbital
α -hydroxymidazolam	diazepam	meperidine	phenytoin
α -hydroxytriazolam	diphenhydramine	mephedrone	propoxyphene
alprazolam	doxepin	meprobamate	propranolol
amitriptyline	doxylamine	methadone	pseudoephedrine
amphetamine	duloxetine	methamphetamine	salicylic acid
benzoylecgonine	EDDP	methanol	secobarbital
butalbital	ephedrine	methylone	sertraline
carbamazepine	ethanol	midazolam	temazepam
carisoprodol	ethylene glycol	mirtazapine	THC
chlordiazepoxide	fentanyl	morphine	THC-COOH
chlorpheniramine	flunitrazepam	nordiazepam	THC-OH
chlorpromazine	fluoxetine	nordoxepin	tramadol
citalopram	flurazepam	norfentanyl	trazodone
clomipramine	gabapentin	norfluoxetine	triazolam
clonazepam	hydrocodone	normeperidine	trimipramine
cocaethylene	hydromorphone	norpropoxyphene	venlafaxine
cocaine	imipramine	nortriptyline	verapamil
	isopropanol	oxazepam	zolpidem

6 Calculations

See the *Guidelines for Toxicological Quantitations* standard operating procedure (Tox 101) for acceptable practices in calculating quantitative results.

7 Measurement Uncertainty

When quantitative results are included in an FBI Laboratory report, the measurement uncertainty

will be estimated and reported following the Chemistry Unit's *Procedures for Estimating Uncertainty in Reported Quantitative Measurements* standard operating procedure (CUQA 13).

8 Limitations

Not applicable.

9 Safety

Chemists and Examiners will follow all safety guidance provided in the *FBI Laboratory Safety Manual*. When opening a blood tube or other biological specimen container, the possibility of aerosolizing the contents exists. In order to prevent unwanted contact with the specimen, several different measures may be taken in addition to wearing appropriate personal protective equipment.

- Specimens may be opened and pipetted in a chemical fume hood.
- Specimens may be opened and pipetted behind a bench top shield.

10 References

Chemistry Unit Quality Assurance and Operations Manual.

FBI Laboratory Operations Manual.

FBI Laboratory Quality Assurance Manual.

FBI Laboratory Safety Manual.

Guidelines for Toxicological Quantitations (Tox 101); FBI Laboratory Chemistry Unit – Toxicology Subunit SOP Manual.

Guidelines for the Toxicological Analysis of Product Tampering Investigations (Tox 105); FBI Laboratory Chemistry Unit – Toxicology Subunit SOP Manual.

Procedures for Estimating Uncertainty in Reported Quantitative Measurement (CUQA 13); FBI Laboratory Chemistry Unit Quality Assurance and Operations Manual.

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Rev. #	Issue Date	History
6	09/28/15	In Sections 1 and 10, updated the reference to the ABFT Checklist. Removed calibration Section (previous Section 4) and renumbered subsequent sections. In Section 4, removed reference to "Qs and Ks". Removed hair from Table 2. Table 3 was updated to account for the replacement of most immunoassays with a UPLC/HRMS method. Updated text in 5.4 to account for newly validated UPLC/HRMS method. Updated Table 4 to account for newly validated analytes. Removed Reagent SOP from Section 10.
7	03/01/16	In Table 2, updated minimum volume of urine. Added guidance for vitreous humor only cases to Section 5.3. Clarified wording in Section 5.4. In Section 9, added guidance for safely opening and pipetting biological specimens.

Approval

Redacted - Signatures on File